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Case Report Open Access

Assessment of Immunoblot for Early Detection of Seroconversion in Pregnant Women

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Abstract

The recently commercialized LDBIO Toxo II IgG® immunoblot test has been shown to be very reliable in the early detection of toxoplasmic seroconversion in pregnant woman. A positive result is defined by the presence of at least three among the five following bands: 30, 31, 33, 40 and 45 kDa, the 30 kDa band being required as a positivity criteria. Moreover, it has been reported that the detection of the sole 30 kDa band was sufficient for this purpose.

We report herein the result of serologic follow-up of three initially seronegative pregnant woman by using ELISA-IgG, IFAT-IgG, ELISA-IgM and LDBIO Toxo II IgG®. In two of them, seroconversion was highly suspected on the detection of 30 kDa band and positivity of IgM test and further confirmed by the positivity of conventional tests for IgG. In contrast, in the third case, despite the appearance of the 30 kDa band during the follow-up, seroconversion was discarded as ELISA-IgG and IFAT-IgG tests remained negative and only one additional band was later detected on LDBIO Toxo II IgG®.

Our findings suggest that the detection of the 30 kDa band together with the positivity of IgM test can be considered as a seroconversion criteria but confirmation by further positivity of conventional tests is recommended.

Keywords: Toxoplasmosis; Seroconversion; Pregnant woman; LDBIO Toxo II IgG® immunoblot

Introduction

Toxoplasmosis is a very common parasitic disease. In immunocompetent individuals, it is usually benign or asymptomatic. However, in the event of pergravidic primary infection, it can cause severe lesions in the fetus because of the risk of placental passage of the parasite [1-3].

The diagnosis of toxoplasmosis is mainly based on the research of specific anti-Toxoplasma antibodies in the serum [4-8]. Serological techniques for the detection and titration of IgG are mainly represented by automated enzyme-linked immunosorbent assay (ELISA) tests. These tests are satisfactory in terms of sensitivity and specificity [9-11]. However, the very low titers and titers close to the cut-off are difficult to interpret and do not allow to formally identify the immune status of patient with respect to Toxoplasma [11-13]. In these situations, the use of a confirmatory technique is highly required. The dye test has long been the gold standard in the diagnosis of toxoplasmosis, but this test is not suitable for routine diagnosis [12,14]. The recently commercialized, LDBIO Toxo II qualitative IgG® immunoblot test (LDBIO Diagnostics, Lyon, France) has been proposed as a very reliable alternative given its excellent concordance with the dye test [12]. In addition, some authors have shown the suitability of this immunoblot test in the early detection of toxoplasmosis seroconversion during pregnancy and its superiority compared to conventional techniques such as ELISA and indirect fluorescent antibody test (IFAT) [11,13]. The aim of the present study was to evaluate and discuss the contribution of the LDBIO Toxo II IgG® immunoblot test in the early detection of seroconversion through three clinical observations of women followed up in the laboratory of Parasitology, Farhat Hached University Hospital, Sousse, Tunisia.

Cases Description

This study included 14 sera collected from three immunocompetent pregnant women with no specific medical background, in the context of routine serological screening for toxoplasmosis and five sera collected from their three neonates in the context of postnatal serological screening.

For the detection of anti-Toxoplasma IgG, the sera were tested by ELISA-IgG (Platelia Toxo IgG®, Bio-Rad, Marnes-la-Coquette, France) and IFAT-IgG (Toxo-Spot IF®, Bio- Mérieux, Marcy l'Etoile, France). The ELISA-IgM (Platelia Toxo IgM®, BioRad, Marnes-la-Coquette, France) was used for the detection of specific IgM. In ELISA-IgG, the results are expressed as international units per milliliter (IU/ml) and their interpretation was based on manufacturer's criteria. The test is regarded positive if ≥ 9.0, negative if <6.0 and equivocal if ≥ 6.0 and <9.0 (gray zone). In ELISA-IgM, results are expressed as positive, negative, or borderline according to the manufacturer's recommendations. The test is considered negative if the ratio (optical density of the tested sample/optical density of the cut-off control) is <0.8, equivocal if ≥ 0.8 and <1.0 (gray zone), and positive if ≥ 1.0. In IFAT-IgG, the titers of sera are calculated by reference to a positive control with a known titer and expressed as IU/ml. The test is positive if ≥ 12.0, negative if <6.0, and equivocal if ≥ 6.0 and <12.0 (gray zone). The LDBIO-Toxo II IgG® immunoblot test (LDBIO, Lyon, France) was carried out according to the manufacturer's guide. Five bands are recognized as specific for *T. gondii* after their revelation with patient

serum. These bands are: 30, 31, 33, 40 and 45 kDa. The molecular weight of these bands is determined by comparison with the molecular weight marker provided by the test and with a positive control, which indicates the precise location of the bands identified in the zone between 30 kDa and 45 kDa. A positive result is defined by the presence of at least three among the five bands, the 30 kDa band being required as a positivity criteria.

For the diagnosis of congenital toxoplasmosis, we tested the serum of the mother and the neonate using the comparative immunoblot test Toxoplasma Western Blot IgG-IgM® (LDBIO Diagnostics, France) according to the manufacturer's recommendations. The presence of one or more additional bands in the serum of the neonate is indicative of congenital toxoplasmosis. Conventional PCR targeting the gene AF172465 (529 base pairs) was used for the detection of Toxoplasma DNA in the amniotic fluid [15].

The first case concerns a 37-year-old pregnant woman which was monitored for Toxoplasma serodiagnosis. The first serology carried out at 12 weeks of amenorrhea (WA) was negative in ELISA-IgG, IFAT-IgG and ELISA-IgM. The second serology performed at 31 WA was negative in both ELISA-IgG and IFAT-IgG but positive in ELISA-IgM (Ratio=2.33). The LDBIO Toxo IgG II® immunoblot carried out on the second serum showed the presence of the single 30 kDa specific band. One week later, we noted the appearance of the 31 kDa, whereas ELISA-IgG and IFAT-IgG remained negative. A fourth serology carried out at 34 WA, was negative in ELISA-IgG, weakly positive in IFAT-IgG (12 IU/ml) and positive in ELISA-IgM (Ratio=7.44). The immunoblot revealed the presence of the 33 kDa in addition to the 30 kDa and 31 kDa bands. The PCR on the amniotic fluid performed at 35 WA was negative, and the mother treated with spiramycin. Echography showed no abnormalities evocative of congenital toxoplasmosis. At delivery (39 WA), the serum of the mother was still negative in ELISA-IgG, positive in IFAT-IgG with the same titer (12 IU/ml) and ELISA-IgM (Ratio=8.28) (Figure 1). The neonate's serum was positive in ELISA-IgG (18 IU/ml) and ELISA-IgM (Ration=5.5). IgG and IgM profiles of the mother and neonate in Toxoplasma Western Blot IgG-IgM® were different showing additional bands on the neonate strip which confirms the diagnosis of congenital toxoplasmosis (Figure 2).

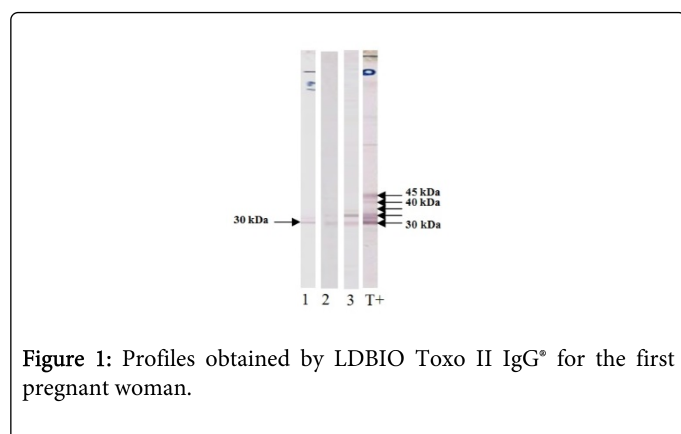

Figure 1: Profiles obtained by LDBIO Toxo II IgG® for the first pregnant woman.

1: at 31 WA*. 2: at 32 WA. 3: at 39 WA (Delivery). T+: Positive control. *Weeks of amenorrhea.

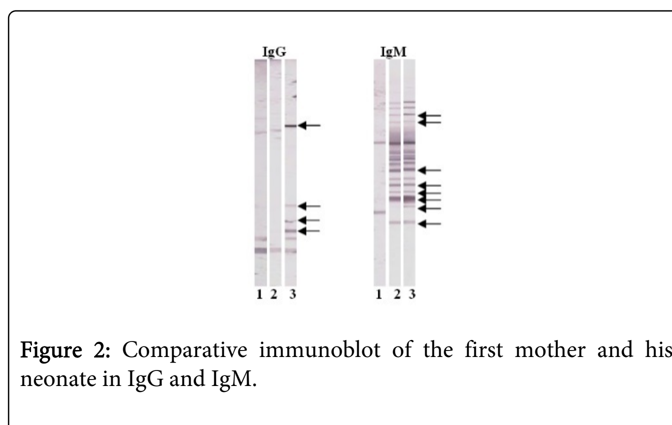

Figure 2: Comparative immunoblot of the first mother and his neonate in IgG and IgM.

1: Mother. 2: Neonate at first day of birth. 3: Neonate at 10th day.

The second case concerns a 27-year-old pregnant woman. The first Toxoplasma serodiagnosis carried out at 24 WA was negative in both ELISA-IgG and IFAT-IgG, but positive in ELISA-IgM (Ratio=3.52). The LDBIO Toxo II IgG® immunoblot showed the specific 30 kDa and 45 kDa bands. Three weeks later, the serum was positive in ELISA-IgG (28 IU/ml), in IFAT-IgG (48 IU/ml) and in ELISA-IgM (Ratio=6.67). The LDBIO Toxo II IgG® revealed the appearance of the 40 kDa band. The mother was treated with spiramycin. At 28 WA, the IgG titer increased in ELISA-IgG (46 IU/ml) and IFAT-IgG (384 IU/ml). The immunoblot profile revealed the presence of the 31 kDa specific band in addition to the 30 kDa and 45 kDa bands. The PCR on the amniotic fluid was positive which confirms the fetal contamination and prompted the replacement of preventive treatment with spiramycin by curative treatment with pyrimethamine-sulfadiazine. At delivery (36 WA), the titer of IgG in IFAT-IgG increased to 768 IU/ml and to 160 IU/ml in ELISA-IgG. ELISA-IgM was positive (Ratio=3.1) and the LDBIO Toxo II IgG® immunoblot revealed the 33 kDa band in addition to the four bands already present (Figure 3). At birth, the serology of the neonate was positive in ELISA-IgG (181 IU/ml) and ELISA-IgM (Ratio=5.8). IgG and IgM profiles of the mother and neonate in Toxoplasma Western Blot IgG-IgM® were different showing additional bands on the neonate strip (Figure 4). This result indicates that the neonate was contaminated.

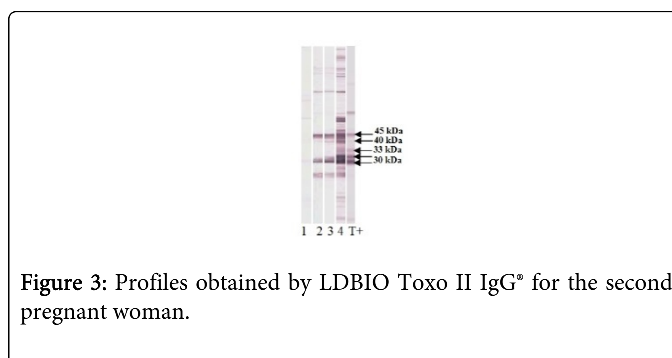

Figure 3: Profiles obtained by LDBIO Toxo II IgG® for the second pregnant woman.

1: at 24 WA*. 2: at 27 WA. 3: at 28 WA. 4: at 36 WA (Delivery). T+: Positive control. * Weeks of amenorrhea.

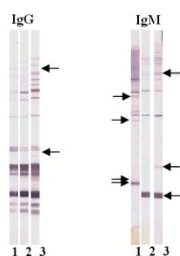

Figure 4: Comparative immunoblot of the second mother and his neonate in IgG and IgM.

1: Mother. 2: Neonate at first day of birth. 3: Neonate at 10th day.

The third case concerns a 33-year-old pregnant woman who was monitored for Toxoplasma serodiagnosis. The first toxoplasmosis serology performed at 15 WA was negative in ELISA-IgG, IFAT-IgG and ELISA-IgM. At the second serology carried out at 17 WA, the ELISA-IgM became positive (Ratio=3.42) while the ELISA-IgG and the IFI-IgG remained negative. The immunoblot LDBIO Toxo II IgG® revealed the single 30 kDa band. A week later, ELISA-IgG and IFAT-IgG were still negative and ELISA-IgM positive (Ratio=5.73), while the LDBIO Toxo II IgG® immunoblot revealed the 30 kDa and 40 kDa bands. The PCR on amniotic fluid performed at 19 WA was negative. The fourth serology carried out at 24 WA gave the same results in ELISA-IgG, IFAT-IgG and ELISA-IgM (Ratio=4.07) and the LDBIO Toxo II IgG® immunoblot showed no additional bands. At delivery (40 WA), the mother serology was negative in ELISA-IgG and IFI- IgG and still positive in ELISA-IgM (Ratio=6.46). However, we noted the disappearance of the 30 kDa band in the LDBIO Toxo II IgG® immunoblot (Figure 5). These findings indicate that the mother was not contaminated. The immunoblot of the mother and the neonate showed identical profiles in IgG and the absence of IgM in the serum of the neonate (Figure 6).

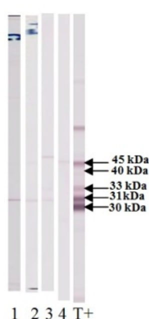

Figure 5: Profiles obtained by LDBIO Toxo II IgG® for the third pregnant woman.

1: à 17 WA*. 2: à 18 WA. 3: à 24 WA. 4: 40 WA (Delivery). T+: Positive control.
*Weeks of amenorrhea.

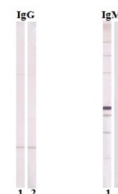

Figure 6: Comparative immunoblot of the third mother and his neonate in IgG and IgM.

1: mother. 2: neonate at first day of birth.

Discussion

Seroconversion in pregnant women needs to be detected as early as possible because of the risk of transmission of Toxoplasma to the fetus. It has been known that treatment when administered as soon as seroconversion is detected significantly minimizes the severity of fetal lesions after transmission has occurred [11,16]. The recently commercialized LDBIO Toxo II IgG® test has been shown to be very reliable in the confirmation of low titers of IgG or titers close to the cut-off of the routine quantitative techniques [12]. Jost et al. reported that the positivity of LDBIO Toxo II IgG® immunoblot test occurs earlier than conventional techniques and that the first band to appear on blot is the 30 kDa band. In addition, they showed that the sole presence of the 30 kDa band, together with positivity of IgM, is very indicative of seroconversion despite the negativity of conventional tests for IgG antibodies, and propose to start spiramycin anti-Toxoplasma treatment as soon as the 30 kDa band is detected [11]. Our findings in the first and second cases are in accordance with the conclusions of Jost et al. as in both cases, seroconversion was confirmed by the further positivity of ELISA-IgG and IFAT-IgG and full positivity of LDBIO Toxo II IgG® test (appearance of additional two and four bands in the first case and second case, respectively), even though in the first pregnant woman, IgG titer remained very low. In this patient, the prescription of spiramycin treatment may have inhibited the increase of IgG titer, in contrast to the second patient where the inhibition doesn't seen to have occurred as IgG titer increased in both ELISA and IFAT tests [2,12]. In addition, our findings confirm that LDBIO Toxo II IgG® test is more sensitive than ELISA-IgG and IFAT-IgG [16].

On the other hand, if the detection of the sole 30 kDa band is considered as a reliable seroconversion criteria, both patients would have benefited of spiramycin treatment three weeks before seroconversion was confirmed by conventional techniques. Considering the 30 kDa band as a criteria of positivity suggests that this band is highly specific. Yet, the detection of this band in negative sera for toxoplasmosis investigated in a previous study was shown to be not uncommon [17]. This indicates that the 30 kDa band may not have an absolute specificity. Indeed, the follow-up of the third patient showed that she was not actually infected despite the detection of the 30 kDa band at first screening. In addition to the absence of the seroconversion of ELISA-IgG and IFAT-IgG tests, only two out of the three bands required for the LDBIO Toxo II IgG® test to be considered positive appeared during the follow-up period. Moreover, the disappearance of the 30 kDa band four months after its first detection may be an additional criteria of the absence of contamination of the

mother. According to these findings, the IgM antibodies detected in the third patient sera are non specific. Anti-Toxoplasma IgM antibodies are usually detected by either ELISA and Immuno-Sorbent Agglutination Assay (ISAGA) techniques. Both tests may expose to false positive results. The specificity of IgM is difficult to check and the only way to confirm this specificity is the detection of IgG in successive sera during follow-up.

To conclude, the first two cases confirm the contribution of LDBIO Toxo II IgG® immunoblot in the early detection of toxoplasmic seroconversion in pregnant women. Nevertheless, as shown by the third case, caution is needed in this situation and further documentation of seroconversion is recommended. In the meanwhile, the patient can be treated with spiramycin in case the contamination is confirmed during further follow-up.

Conflict of Interest

The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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